UNITED STATES DEPARTMENT OF AGRICULTURE
U.S. Agricultural Marketing Service

FURTHER DEVELOPMENTS IN THE PHOTOMETRIC DETERMINATION OF WHEAT PROTEIN

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INTRODUCTION

The photometric method for determining the protein content of wheat flour reported last year by one of us 1/ has been subjected to further study by the Agricultural Marketing Service in an effort to develop a practical method, simpler and more convenient than the established Kjeldahl procedure, for determining protein in wheat. If the method could be developed in such a way that it would differentiate between the gluten proteins of the endosperm and the non-gluten proteins of the non-endosperm constituents of the wheat kernel, its value would be greatly enhanced.

The method is based on the peptization of the wheat proteins by dilute alkali, the preparation of a stable colloidal suspension of the gluten proteins by accurately controlled partial neutralization of the alkaline extract, and the measurement of the light transmission through this suspension by means of the photoelectric photometer.

The proteins of ground wheat are readily peptized by dilute alkali. Neutralization of a portion of the alkaline extract to a definite pH by means of a suitable standard buffer solution causes a marked aggregation of the protein micelles of the prolamines and glutelins into particles sufficiently large to produce a marked turbidity but not large enough to cause precipitation within a reasonable length of time. Properly prepared suspensions of this type are remarkably stable and show very little change in optical density over a period of 24 hours. Globulins, albumins, proteoses, peptides, and amino acids remain completely dispersed under this treatment and, hence, do not contribute appreciably to the turbidity of the suspension.

1/ Zeleny, L., Cereal Chem. 18, 86-92 (1941).

Although wheat germ contains about 40 percent of protein, the protein consists chiefly of a globulin and an albumin, neither of which is determined by the photometric method. Bran contains about 17.5 percent protein consisting of globulin and albumin in addition to considerable quantities of a prolamine and probably a glutelin. It might be expected that the latter two proteins could not be differentiated photometrically from the gluten proteins, but tests on relatively pure bran indicate that these proteins produce considerably less turbidity under the conditions of the test than do the corresponding proteins (the gluten proteins) of the endosperm. It may be stated, then, that the photometric method does differentiate in a large measure between the gluten and the non-gluten proteins of the wheat and that the test is nearly, although not entirely, specific for the gluten proteins.

THE METHOD

The photometric method used in this study was found to be applicable to both wheat and wheat flour. The details of the procedure are given below. This method differs in certain minor respects from the one previously reported for wheat flour (see footnote 1).

(1) To exactly 0.5 gram of the freshly and finely ground, well-mixed sample in a 130 ml. centrifuge tube that can be stoppered, add 100 ml. of 0.05 N. KOH solution.

(2) Shake the stoppered tube intermittently for about 3 minutes, remove the stopper, and centrifuge for 10 minutes at approximately 1800 r.p.m. (In the case of flour lumps may form which must be completely broken up during the shaking process before centrifuging.)

(3) To exactly 5 ml. of the centrifugate in a photometer test tube (one of the selected tubes for use in lieu of an absorption cell), add exactly 25 ml. of a buffer solution made by mixing 6 parts by volume of 0.2 M.KH_2PO_4 with with 94 parts by volume of 0.2 M.Na_2HPO_4. This buffer solution should have a pH of 7.8 and should be preserved by the addition of 1 ml. of toluene per liter of solution. Mix the contents of the test tube by inversion and allow to stand for 1 hour.

(4) Determine the transmission of light through the solution in the test tube with a photoelectric photometer, using a light filter having a maximum transmission at a wave length of 530 millimicrons. (Other wave lengths will give different but equally satisfactory results.)

DETERMINATION OF ENDOSPERM PROTEIN

Since the readings obtained by the photometer presumably are dependent upon the gluten protein rather than upon the total protein content of the sample and since the ratio of gluten protein to total protein varies widely among different samples of wheat, an accurate evaluation of the reliability of the photometric results as a measure of gluten protein can be made only by a direct comparison of photometric readings with gluten content values.

Unfortunately, however, there appears to be no direct and accurate method for determining the gluten protein content of wheat other than the extremely tedious and impractical procedure of dissecting the pure endosperm from a large number of kernels and carrying out peptization studies on the dissected material. In the absence of a practical direct method we have endeavored to calculate the endosperm protein content of wheat indirectly from the total protein and ash content of the sample. Although pure endosperm probably contains proteins other than the gluten proteins it is assumed that the content of such non-gluten protein in the endosperm is very small.

If the ash content of endosperm and the non-endosperm portion of a sample of wheat were known, the percentages of endosperm and non-endosperm constituents could be calculated by simple proportion using the equations:

$$(1) \qquad N = \frac{100\ (A_W - A_E)}{A_N - A_E}$$

$$(2) \qquad E = 100 - N$$

where N = % non-endosperm,
E = % endosperm,
A_W = % ash in wheat,
A_E = % ash in endosperm,
and A_N = % ash in non-endosperm

Since pure bran and pure germ contain close to 9 percent and 5 percent of ash respectively and since the ratio of bran to germ in the wheat kernel is ordinarily about 13:2.5, the ash content of the non-endosperm portion of the wheat kernel is calculated to be approximately 8.4 percent. If it is assumed that the ash content of pure wheat endosperm is 0.35 percent, equation (1) may be simplified to:

$$(3) \qquad N = \frac{100\ (A_W - 0.35)}{8.05}$$

Although the values for ash content of the endosperm and non-endosperm may vary from those used in equation (3), the difference between the two values is so great that the normal variability of either of them will have but little effect on the calculated value of N.

In the general run of hard red winter wheat the ratio of bran weight to germ weight may be considered to be reasonably constant, the weight of endosperm being the moist variable factor. The range in wheat ash ordinarily encountered corresponds to a range in the non-endosperm constituents of from 10 to 30 percent as calculated from equation (3), and the range in germ content is generally considered to be from 2 to 3 percent of the wheat. Using these values, the germ content and the bran content of any sample may be calculated from the non-endosperm content by means of the following equations:

$$G = .05N + 1.5 \quad (4)$$

$$B = N - G \quad (5)$$

Where G = % germ
B = % bran
and N = % non-endosperm (from equation 3)

Since pure bran is said to contain approximately 17.5 percent protein and pure germ 40 percent protein, the bran and germ protein contents of the original wheat may be determined by using the values obtained in equations (4) and (5). The endosperm protein is then obtained by subtracting the sum of the bran and germ protein from the total protein value.

The above method for determining the endosperm protein content of wheat is based on the assumption that the variation in ash content among different samples of wheat is due to the variation in the proportions of the three major structures of the wheat kernel, endosperm, germ, and bran, and not to a variation in the ash content of the individual structures themselves. It is also based on the assumption that variation in wheat protein is due primarily to variation in relative size and protein content of the endosperm, and that the protein content of pure bran and of pure germ is relatively constant. Obviously these assumptions are not strictly true and the calculated values for endosperm protein cannot, therefore, be considered highly accurate. Except in unusual instances, however, it is not likely that variabilities in the values assumed to be constant are sufficiently great to cause serious errors in this method of calculation.

EXPERIMENTAL WORK

One hundred and ninety-five samples of commercial hard red winter wheat of the 1940 crop from 15 different states were analyzed by the photometric method and the light transmission values compared with the protein content values determined by the Kjeldahl method. The results are listed in table 1 and are shown graphically in figure 1.

From a study of the graphical presentation of these data (fig. 1), it became evident that in the case of wheats that were characterized by low test weight, high ash content, and a shriveled appearance, light transmission values usually indicated lower protein contents than those shown by Kjeldahl analysis. Such wheats contain more bran and less endosperm than normal wheats and consequently show a lower than average ratio of gluten protein to total protein. Conversely in the case of wheats that were characterized by high test weight, low ash content, and a plump appearance, light transmission

values usually indicated higher protein contents than those shown by Kjeldahl analysis. Wheats of this type contain less bran and more endosperm than average wheats and hence show a higher than average ratio of gluten protein to total protein. These observations support the theory that light transmission values are a better index of gluten protein than of total protein content.

Approximate values of endosperm protein content for the series of samples under investigation were determined by the method herein described. These values also are listed in table 1 and are shown graphically in figure 2. A comparison of figures 1 and 2 indicates that the endosperm protein appears to bear a linear relationship to light transmission but that the relationship between total protein and light transmission is curvilinear. For the purposes of statistical analysis it was found that this latter relationship could be made linear by using the arcsine of the total protein content rather than the total protein content itself. 2/

The following statistical values for correlation coefficient and standard errors of estimate were obtained:

For total protein and light transmission

$r = -0.969$

$S =$ 0.634 arcsine of the total protein percentage

$=$ approximately 0.49% total protein

For endosperm protein and light transmission

$r = -0.987$

$S =$ 0.44% endosperm protein

The further observation was made that in the case of the endosperm protein data (fig. 2) 76 percent of the values fell within 0.5 percent (in terms of endosperm protein) of the regression line, while in the case of the total protein data (fig. 1), only 62 percent of the values fell within 0.5 percent of the theoretical curve representing the relationship between total light transmission and total protein. Thus the light transmission values are shown to be a somewhat better measure of endosperm protein (and therefore presumably of gluten protein) than of total protein content. This observation lends further support to our theoretical contention.

2/ For a discussion of this technic see Snedecor, G. W., Statistical Methods, Ed. 3, p. 382, (1940). The Iowa State College Press.

Table 1.- Total protein, ash, endosperm protein (calculated), and light transmission values on 195 samples of hard red winter wheat from the 1940 crop. Samples are listed in order of increasing total protein content. All data are on a "as is" moisture basis

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		Percent	Percent	Percent	Percent
137	Idaho	8.23	1.47	5.30	76.5
455	Washington	8.28	1.62	5.00	75.2
439	Washington	8.38	1.35	5.72	72.2
621	Washington	8.53	1.62	5.25	73.4
540	Montana	8.62	1.76	5.00	76.3
1,000	Indiana	8.66	1.82	4.92	73.0
614	Washington	8.73	1.48	5.77	70.3
139	Idaho	8.80	1.72	5.27	72.9
145	Washington	8.96	1.68	5.54	73.2
147	Idaho	9.04	1.54	5.95	73.0
148	Washington	9.05	1.62	5.77	72.6
858	Montana	9.06	1.77	5.42	73.5
479	Washington	9.10	1.68	5.86	71.8
433	Washington	9.33	1.46	6.41	69.9
480	Idaho	9.38	1.78	5.72	69.5
489	Idaho	9.38	1.76	5.76	69.5
163	Washington	9.43	1.58	6.22	69.3
451	Montana	9.48	1.68	6.06	70.3
486	Washington	9.50	1.73	5.95	69.6
475	Washington	9.52	1.62	6.24	68.7
985	Indiana	9.53	1.75	5.94	69.8
806	Kansas	9.53	1.74	5.96	71.5
143	Washington	9.53	1.61	6.28	69.9
443	Washington	9.55	1.70	6.09	69.3
173	Washington	9.64	1.60	6.39	68.2
155	Washington	9.64	1.39	6.91	67.9
628	Washington	9.68	1.65	6.33	71.3
456	Washington	9.68	1.58	6.47	71.1
135	Washington	9.70	1.61	6.45	70.5
171	Washington	9.71	1.62	6.43	69.8
437	Washington	9.75	1.47	6.82	70.5
129	Washington	9.76	1.79	6.09	72.6
141	Washington	9.77	1.64	6.45	69.5
612	Washington	9.78	1.49	6.80	68.2
854	Montana	9.79	1.70	6.33	69.8
374	Illinois	9.79	1.68	6.37	70.1
631	Washington	9.81	1.48	6.85	68.6
467	Washington	9.86	1.58	6.65	70.2
385	Illinois	9.87	1.77	6.23	69.4
453	Washington	9.92	1.42	7.10	68.1
436	Washington	9.93	1.59	6.72	66.7

Continued

Table 1.- Total protein, ash, endosperm protein (calculated), and light transmission values on 195 samples of hard red winter wheat from the 1940 crop. Samples are listed in order of increasing total protein content. All data are on an "as is" moisture basis

Continued

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		Percent	Percent	Percent	Percent
617	Washington	9.93	1.46	7.01	67.1
472	Washington	9.96	1.45	7.07	69.3
174	Idaho	9.97	1.79	6.30	70.0
161	Washington	10.04	1.35	7.38	67.2
447	Oregon	10.05	1.95	6.00	70.0
444	Washington	10.07	1.59	6.86	70.6
756	Kansas	10.11	1.56	6.99	68.5
694	Illinois	10.25	1.89	6.33	71.3
718	Illinois	10.35	1.93	6.34	69.9
478	Washington	10.42	1.50	7.42	65.9
672	Illinois	10.46	1.71	6.98	69.4
857	Minnesota	10.57	2.08	6.20	66.5
998	Indiana	10.62	1.87	6.77	67.9
562	Kansas	10.62	1.77	6.98	68.3
997	Indiana	10.67	1.84	6.87	68.9
673	Illinois	10.70	1.81	6.97	68.5
737	Kansas	10.89	1.49	7.91	64.5
675	Illinois	11.10	1.84	7.30	66.8
715	Illinois	11.22	1.87	7.37	68.2
671	Illinois	11.23	1.74	7.66	65.5
595	Kansas	11.27	1.78	7.61	64.8
609	Kansas	11.32	1.76	7.70	63.2
681	Illinois	11.32	1.71	7.84	68.4
683	Missouri	11.38	1.87	7.53	66.7
674	Illinois	11.41	1.81	7.68	66.9
682	Illinois	11.46	1.83	7.70	65.6
984	Illinois	11.47	1.81	7.74	64.2
600	Kansas	11.52	2.02	7.32	64.5
865	Iowa	11.52	1.86	7.69	68.7
844	Montana	11.56	1.67	8.15	62.9
680	Illinois	11.58	1.77	7.94	65.0
869	Iowa	11.59	1.88	7.72	64.4
702	Kansas	11.60	1.66	8.23	62.3
729	Kansas	11.61	1.59	8.40	63.1
676	Oklahoma	11.72	1.78	8.06	63.8
748	Oklahoma	11.77	1.85	7.96	65.2
866	Iowa	11.80	2.05	7.54	64.4
592	Kansas	11.80	1.65	8.45	61.5
678	Illinois	11.83	1.78	8.17	63.9

Continued

Table 1.- Total protein, ash, endosperm protein (calculated), and light transmission values on 195 samples of hard red winter wheat from the 1940 crop. Samples are listed in order of increasing total protein content. All data are on an "as is" moisture basis

Continued

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		Percent	Percent	Percent	Percent
1,001	Iowa	11.91	1.62	8.63	62.2
571	Nebraska	11.92	1.74	8.35	63.8
632	Oregon	12.03	1.77	8.39	63.8
584	Kansas	12.04	1.66	8.67	60.8
604	Kansas	12.04	1.57	8.86	61.4
665	Oregon	12.07	1.76	8.45	62.9
579	Kansas	12.12	1.77	8.48	63.6
961	Oklahoma	12.12	1.78	8.46	61.8
846	Montana	12.21	1.57	9.03	62.3
606	Kansas	12.28	1.88	8.41	60.0
599	Kansas	12.30	1.63	9.00	59.9
589	Nebraska	12.40	1.81	8.67	61.2
711	Kansas	12.42	1.84	8.62	60.8
835	Iowa	12.47	1.76	8.85	61.4
567	Kansas	12.48	1.70	9.02	61.0
1,086	Texas	12.54	1.73	8.99	62.5
789	Iowa	12.71	1.89	8.79	62.2
561	Kansas	12.75	1.78	9.09	59.9
629	Montana	12.76	1.58	9.55	61.0
825	Iowa	12.81	1.92	8.82	61.6
713	Oklahoma	12.86	1.76	9.24	59.2
588	Oklahoma	12.87	1.80	9.18	59.0
620	Montana	12.91	1.62	9.63	59.2
828	Iowa	12.92	1.96	8.86	61.7
568	Kansas	13.26	1.63	9.96	57.4
586	Kansas	13.37	1.78	9.71	57.9
861	Iowa	13.49	1.77	9.85	58.3
952	Oklahoma	13.62	1.69	10.18	55.1
1,031	Iowa	13.62	1.71	10.14	55.9
686	Oklahoma	13.68	1.61	10.43	56.0
692	Oklahoma	13.73	1.88	9.86	56.8
630	Montana	13.76	1.49	10.78	56.6
940	Oklahoma	13.87	1.74	10.30	57.8
573	Kansas	13.89	1.81	10.16	58.4
685	Oklahoma	13.97	1.82	10.23	54.4
615	Montana	14.09	1.46	11.17	53.7
623	Montana	14.11	1.50	11.11	56.7
618	Montana	14.16	1.56	11.04	53.8
613	Montana	14.26	1.39	11.53	53.4
785	Nebraska	14.32	1.76	10.70	55.5

Continued

Table 1.- Total protein, ash, endosperm protein (calculated), and light transmission values on 195 samples of hard red winter wheat from the 1940 crop. Samples are listed in order of increasing total protein content. All data are on an "as is" basis

Continued

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		Percent	Percent	Percent	Percent
684	Oklahoma	14.37	1.92	10.38	56.9
873	Wyoming	14.37	1.70	10.91	56.7
622	Montana	14.47	1.67	11.06	53.9
791	Nebraska	14.51	1.84	10.71	56.7
605	Kansas	14.51	1.73	10.96	52.5
783	Nebraska	14.56	1.88	10.69	56.9
788	Nebraska	14.56	1.77	10.92	52.6
601	Kansas	14.56	1.65	11.21	55.7
624	Montana	14.69	1.58	11.48	52.2
627	Montana	14.71	1.59	11.50	57.2
790	Nebraska	14.78	1.75	11.19	55.3
559	Nebraska	14.80	1.78	11.14	53.9
578	Kansas	14.87	1.89	10.95	54.3
616	Montana	14.88	1.35	12.22	49.2
784	Nebraska	14.92	1.92	10.93	56.2
843	Montana	14.96	1.66	11.59	51.7
572	Kansas	15.06	1.76	11.44	56.4
574	Oklahoma	15.12	1.76	11.55	51.3
677	Oklahoma	15.13	1.89	11.21	52.2
564	Nebraska	15.20	1.77	11.56	53.3
747	Texas	15.21	1.86	11.38	52.1
786	Nebraska	15.34	1.79	11.67	52.2
745	Texas	15.35	2.02	11.15	52.6
679	Texas	15.36	1.99	11.23	50.2
587	Kansas	15.56	2.06	11.25	54.6
602	Nebraska	15.56	1.98	11.44	53.3
590	Kansas	15.58	1.83	11.82	50.4
576	Kansas	15.66	1.65	12.31	50.8
619	Montana	15.66	1.47	12.73	50.7
734	Oklahoma	15.69	1.73	12.14	52.3
591	Nebraska	15.71	1.70	12.25	49.0
625	Montana	15.74	1.59	12.53	49.9
913	Wyoming	15.76	1.69	12.32	50.7
585	Kansas	16.09	1.86	12.26	50.6
607	Kansas	16.31	2.17	11.77	51.2
691	Oklahoma	16.38	1.71	12.90	48.6
603	Kansas	16.41	2.01	12.22	50.3
693	Oklahoma	16.51	1.86	12.68	49.1

Continued

Table 1.- Total protein, ash, endosperm protein (calculated), and light transmission values on 195 samples of hard red winter wheat from the 1940 crop. Samples are listed in order of increasing total protein content. All data are on an "as is" moisture basis

Continued

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		Percent	Percent	Percent	Percent
626	Montana	16.51	1.61	13.26	51.5
582	Kansas	16.54	2.19	11.95	52.6
593	Nebraska	16.63	2.04	12.39	49.4
575	Kansas	16.76	2.25	12.02	49.9
580	Nebraska	16.81	2.19	12.22	51.0
849	Colorado	16.83	1.94	12.80	48.7
594	Kansas	16.84	1.83	13.08	48.9
610	Montana	16.91	1.61	13.66	48.7
577	Kansas	16.94	2.10	12.54	48.3
787	Nebraska	16.99	1.94	12.96	48.8
1,085	Texas	17.00	1.88	13.13	46.9
896	Wyoming	17.16	1.89	13.24	50.3
1,078	Texas	17.36	2.03	13.14	45.5
797	Kansas	17.39	2.22	12.70	47.7
583	Kansas	17.40	2.14	12.91	50.1
581	Nebraska	17.41	2.06	13.10	48.5
565	Kansas	17.47	2.03	13.25	48.0
569	Kansas	17.68	2.21	13.03	46.1
560	Nebraska	17.74	2.18	13.18	49.5
608	Nebraska	17.91	2.24	13.19	45.7
598	Kansas	17.98	2.25	13.24	48.1
570	Kansas	17.99	2.18	13.43	48.5
527	Colorado	18.03	2.17	13.49	45.8
566	Nebraska	18.04	2.03	13.82	45.9
543	Kansas	18.15	2.33	13.25	47.6
596	Kansas	18.19	2.08	13.82	45.2
518	Kansas	18.22	2.29	13.39	46.5
563	Kansas	18.32	2.49	13.03	47.8
597	Kansas	18.32	2.25	13.58	47.4
58	Kansas	18.36	2.34	13.45	46.1
28	Kansas	18.45	2.32	13.57	43.3
23	Kansas	18.48	2.15	13.97	44.2
72	Kansas	18.65	2.33	13.75	43.6
11	Kansas	18.65	2.32	13.77	43.3
84	Kansas	18.71	2.26	13.94	45.2
40	Kansas	18.76	2.54	13.39	47.1
59	Kansas	18.82	2.13	14.35	44.5

Summary

The photometric method previously reported for the determination of protein in wheat flour has been applied, with minor modifications, to the analysis of wheat. Theoretically the method differentiates to a considerable degree between gluten and non-gluten protein and thus comes closer to being a measure of gluten protein than of total protein. Experimental evidence substantiating this theory is presented.

The principal advantages of the photometric method for routine protein analysis should be the ease and rapidity with which a large volume of work can be handled with relatively simple equipment and without the unpleasant features usually associated with a protein laboratory. An additional advantage is that the results obtained are probably a somewhat better index of ultimate baking quality than are the values obtained by the conventional Kjeldahl procedure.

Further work will be directed toward the adequate standardization of the method and of the photometers used in order that concordant results may be obtained among different laboratories.

ACKNOWLEDGEMENT

The assistance of H. C. Fellows and Louis Feinstein of the Agricultural Marketing Service, who made the Kjeldahl protein determinations, and of O. E. Rauchschwalbe of the Bureau of Plant Industry who conducted the statistical analysis of the data is gratefully acknowledged.

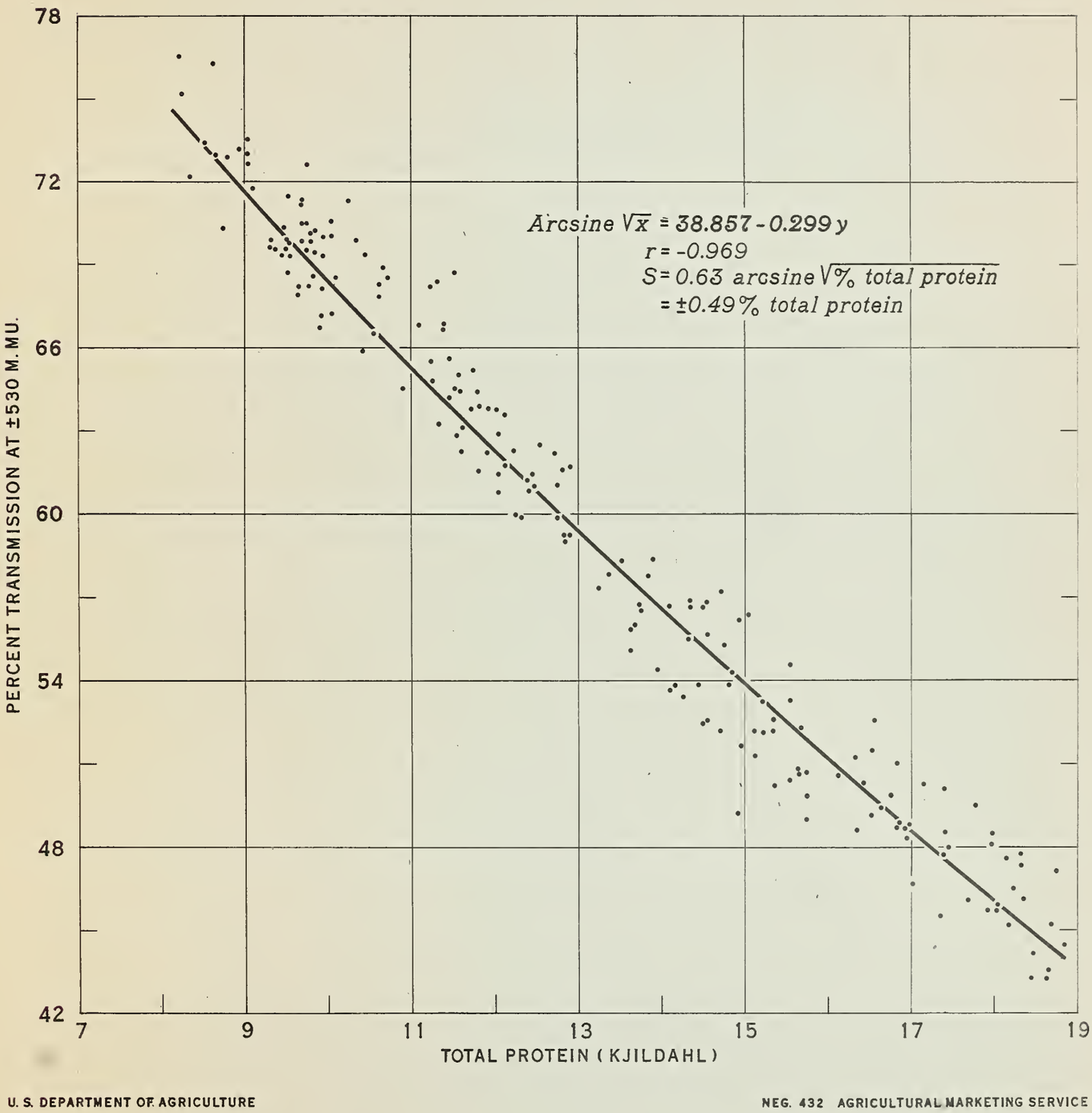

FIGURE 1.- TOTAL PROTEIN AND LIGHT TRANSMISSION VALUES ON 195 SAMPLES OF HARD RED WINTER WHEAT.

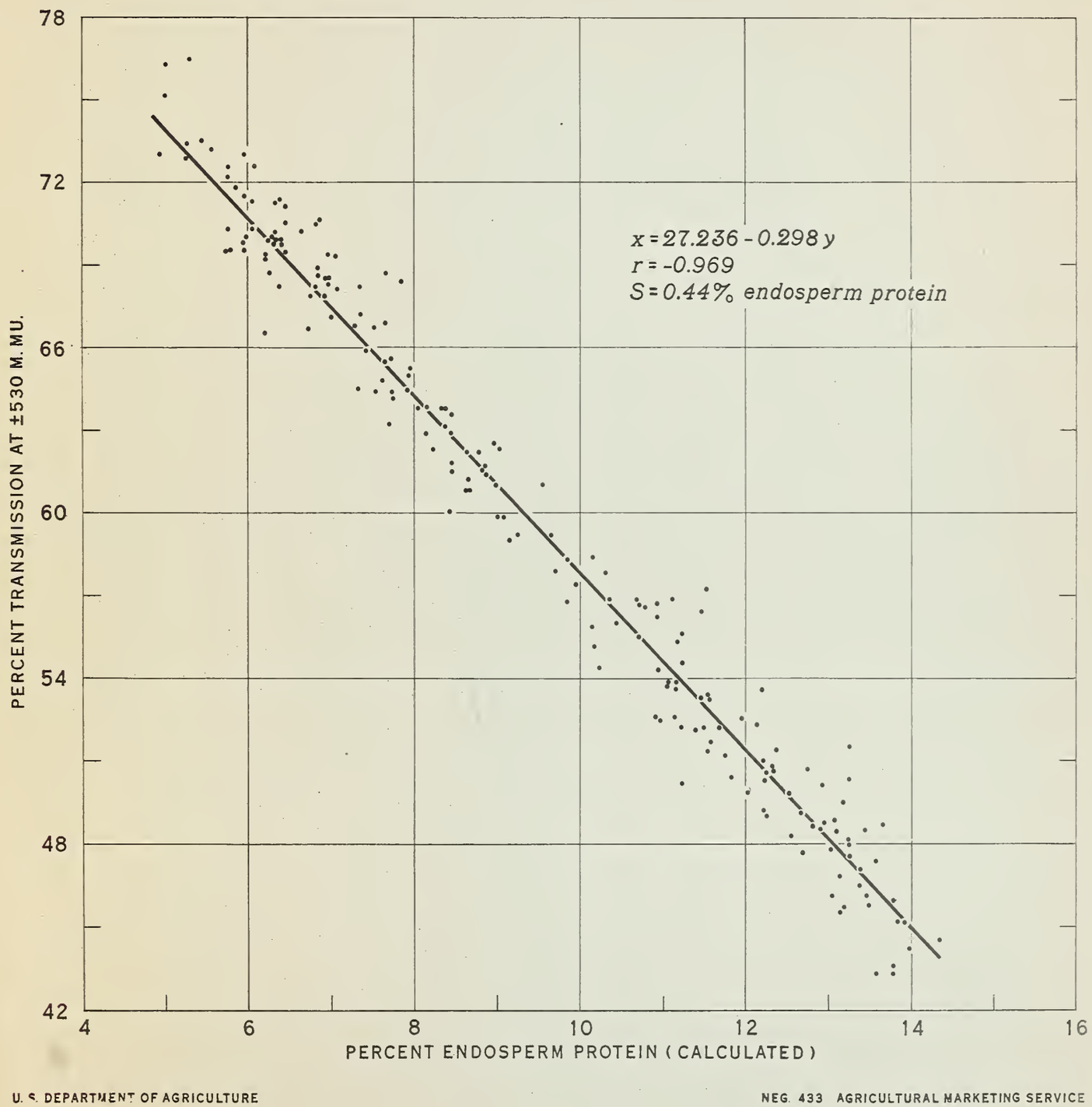

FIGURE 2.- ENDOSPERM PROTEIN (CALCULATED) AND LIGHT TRANSMISSION VALUES ON 195 SAMPLES OF HARD RED WINTER WHEAT.